Genotypic Identification and Phylogenetic Characterisation of Yeasts from Austrian Dairy Products

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Milk and dairy products are important habitats for yeasts. The yeasts are considered as spoilage organisms, but they can play an essential role in processing and ripening of certain cheeses and fermented products. It is, therefore, of value to know which yeasts are associated with a particular product in its natural state and which of the yeasts do not belong to the typical microbial flora. The information about the yeast species is important to detect the origin of the contamination, or to estimate the microbiological quality of a product. Different methods were used over the years to identify yeasts at the species level. Traditional identification methods, based on morphological, biochemical and physiological criteria, are time consuming, lack discriminatory power and misidentification occurs frequently.

A progress in molecular biology in the last decade opened the possibilities to characterise yeasts at the genomic level. The PCR-mediated techniques for yeast typing are already established (e.g. COUTO et al., 1994; MESSNER et al., 1994; MOLNAR et al., 1995, 1996, LOPANDIC et al., 1996, PRILLINGER et al., 1997, 1999). Determination of individual genes coding for 18S and 26S ribosomal RNA (rRNA) brought about many changes in the identification and classification of yeasts (e.g. KURTZMAN and ROBNETT, 1998, FELL et al., 2000).

The rRNA genes were sequenced in several hundreds of yeast strains and these sequences are available from the databank at NCBI (http://www.ncbi.nlm.nih.gov), EMBL (http://www.embl-heidelberg.de) or DDBJ (http://www.ddbj.nig.ac.jp).

In the present work a computer-assisted identification based on the phenotypic and genotypic characterisation and information available from the GenBank was employed to characterise and identify yeast microflora from different milk products (cheeses, yoghurt, butter, sour cream, whey).

Predominant part (461 strains) of the yeast isolates was represented by ascomycetous yeasts, whereas 53 isolates were of basidiomycetous origin. The yeast strains were characterised by phenotypic markers and partial sequencing of 26S rRNA coding gene.

The results of the physiological investigation were evaluated with a computer identification program (Barnett, 1990) to obtain the yeast species that most nearly match the entered data set. The strains with highest identity score were chosen as type strains in the RAPD-PCR analysis. On the other hand, the determined 26S rDNA sequences of the yeast isolates from milk products were imported into the Genbank and by means of the BLAST search program, the strain with the homologous sequence was selected. The best matching sequence for all investigated yeasts were retrieved from the Genbank and included in the phylogenetic analysis.

The determined closely related species for every yeast isolate was used in the RAPD-PCR analysis.

The overall investigation has shown that 473 (92%) yeast strains belong to the following 27 species: *Candida catenulata*, *C. incospicua*, *C. intermedia*, *C. parapsilosis*, *C. pararugosa*, *C. pseudoglaebosa*, *C. saitoana*, *C. sake*, *C. sojae*, *C. zeylanoides*, *Clavispora lusitaniae*, *Debaryomyces fabryi*, *D. hansenii*, *Geotrichum candidum*, *Issatchenkia orientalis*, *Kluyveromyces lactis*, *K. marxianus*, *Pichia fermentans*, *P. guilliermondii*, *Saccharomyces cerevisiae*, *S. unisporus*, *Torulaspora delbrueckii*, *Yarrowia lipolytica*, *Cryptococcus curvatus*, *Rhodotorula mucilaginosa*, *Trichosporon cutaneum* and *T. ovoides*.

The remaining strains (8%) were characterised on the genus level. To get better insight to their phylogenetic relationship with asco- and basidiomycetous yeasts, complete sequences of the 18S rRNA coding gene were estimated. The phylogenetic studies based on the two individual genes as well as the RAPD-analysis indicated that new species of the genera *Yarrowia*, *Rhodotorula*, *Cryptococcus* and *Trichosporon* can be defined.

The results of the present work have shown that the traditional phenotypic characterisation of yeasts is of limited potential. Only 53% of all investigated strains were identified reliably using morphological, biochemical and physiological criteria.

Molecular characterisation based on the DNA-typing techniques, sequence analysis of the rRNA genes and phylogenetic studies is an excellent alternative approach compared to the time consuming physiological tests. On-line access to the data bases as well as existence of the resource centre, such as the ”in-house” yeast collection of the Institute of Applied Microbiology, with approx. 3000 ascomycetous and basidiomycetous strains, are prerequisites for a reliable and fast characterisation and identification.

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